

Soluble Oligomers of Amyloid β Protein Facilitate Hippocampal Long-Term Depression by Disrupting Neuronal Glutamate Uptake

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SUMMARY

In Alzheimer's disease (AD), the impairment of declarative memory coincides with the accumulation of extracellular amyloid- β protein (A β) and intraneuronal tau aggregates. Dementia severity correlates with decreased synapse density in hippocampus and cortex. Although numerous studies show that soluble A β oligomers inhibit hippocampal long-term potentiation, their role in long-term synaptic depression (LTD) remains unclear. Here, we report that soluble A β oligomers from several sources (synthetic, cell culture, human brain extracts) facilitated electrically evoked LTD in the CA1 region. A β -enhanced LTD was mediated by mGluR or NMDAR activity. Both forms of LTD were prevented by an extracellular glutamate scavenger system. A β -facilitated LTD was mimicked by the glutamate reuptake inhibitor TBOA, including a shared dependence on extracellular calcium levels and activation of PP2B and GSK-3 signaling. In accord, synaptic glutamate uptake was significantly decreased by soluble A β . We conclude that soluble A β oligomers perturb synaptic plasticity by altering glutamate recycling at the synapse and promoting synapse depression.

INTRODUCTION

Alzheimer's disease (AD), the most common neurodegenerative disorder, is characterized by progressive memory and cognitive impairment and cerebral accumulation of extracellular amyloid plaques and intraneuronal neurofibrillary tangles. Although the specific molecular initiators of AD remain unknown in most patients, extensive research suggests that the amyloid β protein (A β) plays an early and essential pathogenic role. Of note, dementia severity in AD correlates more strongly with cortical levels of soluble A β species than with insoluble amyloid plaque burden (Lue et al., 1999; McLean et al., 1999). Experimentally, soluble A β oligomers have been specifically shown to block hippocampal long-term potentiation (LTP), an electrophysiological correlate of learning and memory (e.g., Lambert et al., 1998; Walsh et al., 2002; Wang et al., 2002; Townsend et al., 2006;

Shankar et al., 2008). In accord, impairment of synaptic plasticity can be detected in vivo before the formation of insoluble A β deposits in APP transgenic mouse models (e.g., Hsia et al., 1999; Mucke et al., 2000). Synthetic A β aggregates have been reported to inhibit N-methyl-D-aspartate receptor (NMDAR)-dependent LTP, but not NMDAR-independent LTP (Chen et al., 2002; Wang et al., 2004a; but see Raymond et al., 2003). This finding is consistent with evidence that A β can affect surface expression of NMDARs (Snyder et al., 2005; Dewachter et al., 2009) and may increase (Molnár et al., 2004; Wu et al., 1995) or decrease (Chen et al., 2002; Raymond et al., 2003) NMDAR conductance.

A principal neuropathological finding in AD subjects is cortical atrophy associated with degeneration of neurites, decreased dendritic spine density, and frank neuronal loss (Terry et al., 1991; Knobloch and Mansuy, 2008). Anatomical studies in normal rodents suggest that the induction of LTP is associated with spine formation and increased spine volume, whereas the induction of long-term synaptic depression (LTD) results in decreased spine volume and spine elimination (Matsuzaki et al., 2004; Nägerl et al., 2004; Zhou et al., 2004; Bastrikova et al., 2008). Similar to LTP, the induction of LTD in the CA1 region of hippocampus requires activation of NMDAR and/or metabotropic glutamate receptors (mGluR), depending on the stimulation protocol and recording conditions (Kemp and Bashir, 2001; Anwyl, 2006; Citri and Malenka, 2008). Mechanistically, synapse potentiation versus depression may ultimately depend on alterations in cytosolic Ca^{2+} concentration and the differential activation of certain kinases and phosphatases, including p38 mitogen-activated protein kinase (MAPK) and calcineurin (protein phosphatase 2B [PP2B]) (reviewed in Kemp and Bashir, 2001; Citri and Malenka, 2008).

Although numerous reports describe the effects of soluble A β species on LTP, only a few studies have examined LTD induction, and they have yielded inconsistent results. For example, synthetic A β peptides were reported to facilitate LTD induction in an NMDAR-dependent manner in vivo (Kim et al., 2001), whereas other studies found no effect on LTD in slices (Raymond et al., 2003; Wang et al., 2002, 2004a). We recently extracted buffer-soluble A β directly from the brains of typical AD patients and showed that this extract, which contains soluble A β dimers and trimers, facilitated LTD induction in the CA1 region of mouse hippocampus by an mGluR-dependent mechanism (Shankar et al., 2008). Given that both NMDAR and mGluR activation have been implicated in the effects of A β on LTD, we asked

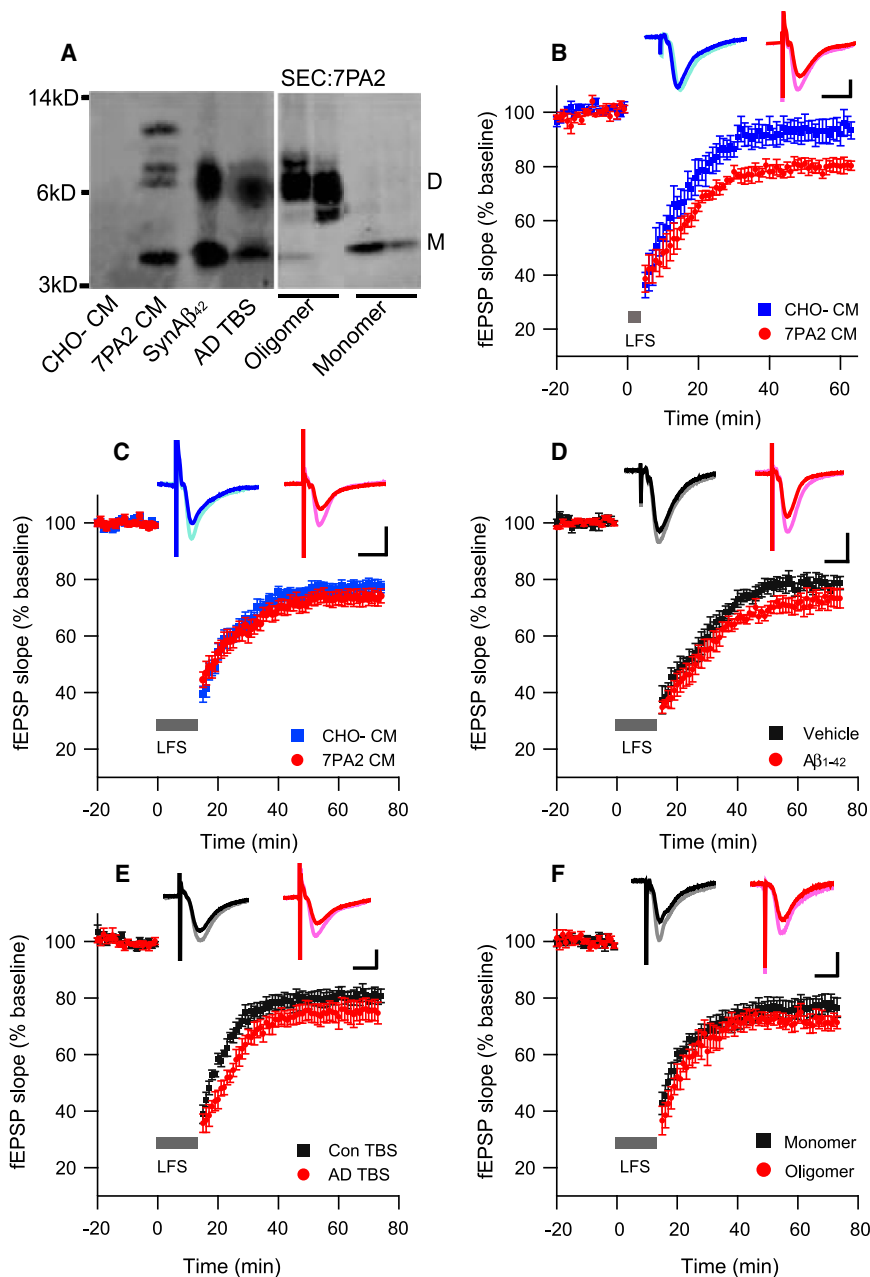


Figure 1. Soluble A β Facilitates Long-Term Depression in the CA1 Region of Hippocampal Slices

(A) Western blot of the three sources of soluble A β used for LTD experiments in this study. All three contain an SDS-stable band at ~8 kDa, and this has been confirmed previously as an A β dimer by mass spectrometry (Shankar et al., 2008). 7PA2 CM also contains a higher, apparent trimer species. CHO- CM is devoid of these human A β species, as expected, and serves as a negative control throughout these studies. All samples were immunoprecipitated with polyclonal A β antibody AW8 and blotted with combined A β monoclonal antibodies 2G3 (A β_{40}) and 21F12 (A β_{42}). SEC: fractions rich in oligomers (left) or monomers (right) separated by SEC of 7PA2 CM.

(B) A train of 300 single pulses at 1 Hz (5 min; small gray bar) did not induce LTD in acute mouse hippocampal slices in the presence of CHO- CM (blue squares, $n = 6$) but induced a significant LTD in the presence of 7PA2 CM (red circles, $n = 7$).

(C–F) A standard LTD protocol of 900 single pulses at 1 Hz (15 min; long gray bar) was applied to slices treated with 7PA2 CM (C), synthetic A β_{1-42} (D), AD brain TBS extract (E), or SEC fractions from 7PA2 CM (F). All are not significantly different from their respective controls. Insets in (B)–(F) represent typical field excitatory postsynaptic potentials (fEPSPs) recorded before (light) and 50 min after (dark) LFS. Horizontal calibration bar, 10 ms; vertical bar, 0.5 mV.

RESULTS

Soluble Human A β from Several Sources Facilitates Hippocampal LTD

Small, soluble A β assemblies have been obtained from a variety of sources, including synthetic peptides, cell culture medium, and human brain extracts (Lambert et al., 1998; Podlinsky et al., 1995; McLean et al., 1999; Shankar et al., 2008). A common feature among the soluble A β preparations used in the current study is the presence of low- n assembly forms,

whether glutamate clearance mechanisms are perturbed by A β . In addition to affecting synaptic plasticity, excitotoxic effects of glutamate are believed to contribute to progressive neuronal loss in AD (Pomara et al., 1992; Harkany et al., 2000). Furthermore, gene expression and protein levels of excitatory amino acid transporters (EAAT1 and EAAT2) are altered in the hippocampus and frontal cortex of AD subjects (Jacob et al., 2007). Here, we provide evidence that small, soluble A β assemblies from several sources enhance synaptic depression through a mechanism involving altered glutamate uptake at hippocampal synapses. Our results have both mechanistic and therapeutic implications for the initiation of hippocampal synaptic failure in AD and in more subtle forms of age-related A β accumulation.

i.e., A β oligomers that are slightly larger than the monomer but much smaller than amyloid fibrils. When examined on denaturing gels, these oligomers run principally as SDS-stable dimers and trimers (Figure 1A). We recently reported that soluble oligomers extracted directly from human (AD) cortex facilitate LTD induction through activation of mGluR when a subthreshold (300 pulses, 1 Hz) stimulation is applied to hippocampal slices (Shankar et al., 2008). To investigate the mechanisms underlying the enhancement of LTD by soluble human A β , we first used cell-secreted A β species obtained from a CHO cell line that stably expresses hAPP with the V717F amyloidogenic AD mutation (7PA2 cells). These cells release readily detectable levels of monomeric and oligomeric A β species into the medium in the

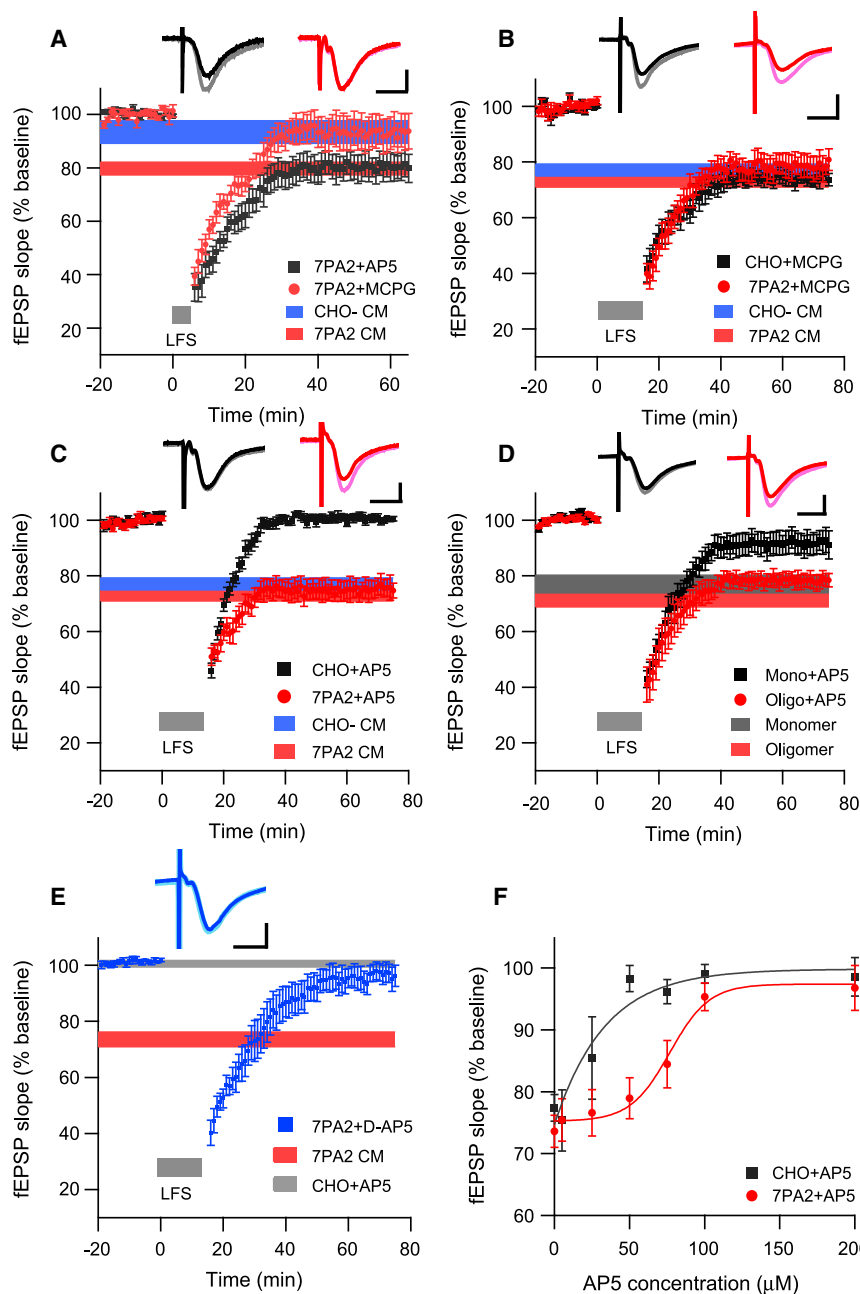


Figure 2. Soluble A β Enhances Hippocampal LTD through mGluR or NMDAR, Depending on the Stimulation Protocol

(A) LTD induced by the 300-pulse protocol (gray bar) in the presence of 7PA2 CM was blocked upon coadministration of the nonselective group I/II mGluR antagonist, MCPG (500 μ M, red circles, $n = 6$), but not the NMDAR antagonist, AP5 (50 μ M, black squares, $n = 5$). Horizontal colored bars represent the corresponding means \pm SEMs from data shown in Figure 1B.

(B) LTD induced by the 900-pulse protocol (gray bar) is independent of mGluR activation. Horizontal colored bars represent the corresponding means \pm SEMs from data shown in Figure 1C.

(C) The 900-pulse LTD induced in slices in CHO- CM was blocked by coadministering the NMDAR antagonist, AP5 (50 μ M, black squares, $n = 8$), whereas LTD in slices in 7PA2 CM was unaltered (red circles, $n = 6$). Horizontal colored bars from data in Figure 1C.

(D) 900-pulse LTD in A β monomer-treated slices was blocked upon coprefusing AP5 (50 μ M, black squares, $n = 5$), whereas the LTD in oligomer-treated slices was not (red circles, $n = 6$). Horizontal colored bars represent the corresponding means \pm SEMs from Figure 1F.

(E) 7PA2 CM enhanced LTD was blocked by treatment with D-AP5 at 100 μ M ($n = 5$). Horizontal colored bars represent the corresponding means \pm SEMs from Figure 2C.

(F) Dose-response curves of LTD blockade by AP5 in either CHO- CM (black squares) or 7PA2 CM (red circles). Data are means \pm SEMs.

Insets in (A)–(E) represent typical fEPSPs recorded before (light) and 50 min after (dark) the LFS. Horizontal calibration bar, 10 ms; vertical bar, 0.5 mV.

absence of insoluble aggregates (Podlisny et al., 1995; Walsh et al., 2002). In accord with our recent work using soluble A β extracted from human cortex, we found that 7PA2 CM (but not the CM of untransfected [CHO-] cells) facilitated the induction of hippocampal LTD following a subthreshold, low-frequency stimulus (LFS) (300 pulses at 1 Hz) that otherwise does not induce LTD ($79.7\% \pm 2.4\%$, $n = 6$, versus $93.4\% \pm 4.2\%$, $n = 7$, of baseline fEPSP slope; $p < 0.05$) (Figure 1B). We next sought to establish the effects of soluble A β on the expression of LTD using a standard LTD-inducing protocol known to be NMDAR dependent (Kemp and Bashir, 2001), namely, 900 pulses at 1 Hz. This form of LTD was equally inducible in slices perfused with artificial

cerebrospinal fluid (ACSF) supplemented with 7PA2 CM or CHO- CM ($73.6\% \pm 2.6\%$, $n = 9$, versus $77.1\% \pm 2.1\%$, $n = 9$; $p > 0.05$) (Figure 1C). Similar results were observed using synthetic A β_{1-42} (Figure 1D), soluble extracts of human AD cortex containing A β oligomers (Figure 1E), or size-exclusion chromatography (SEC) fractions of 7PA2 CM enriched in either oligomers or monomers (Figures 1A, right panel, and Figure 1F). These data are consistent with

previous reports that synthetic A β does not significantly change the magnitude of NMDAR-dependent (i.e., 900-pulse) LTD achieved in vitro (Raymond et al., 2003; Wang et al., 2002, 2004a). To probe the mechanism of A β -facilitated LTD, we used the nonselective group I/II mGluR antagonist MCPG (500 μ M) and the NMDAR antagonist AP5 (50 μ M). In agreement with our previous finding using soluble A β from AD cortex (Shankar et al., 2008), the facilitation of 300-pulse LTD by 7PA2 CM was dependent on activation of mGluR but not NMDAR, as the LTD was MCPG sensitive and AP5 resistant ($93.2\% \pm 6.1\%$, $n = 6$, versus $80.4\% \pm 4.8\%$, $n = 5$; $p < 0.05$) (Figure 2A). Unlike the 300-pulse LTD, 900-pulse LTD induced in the presence of

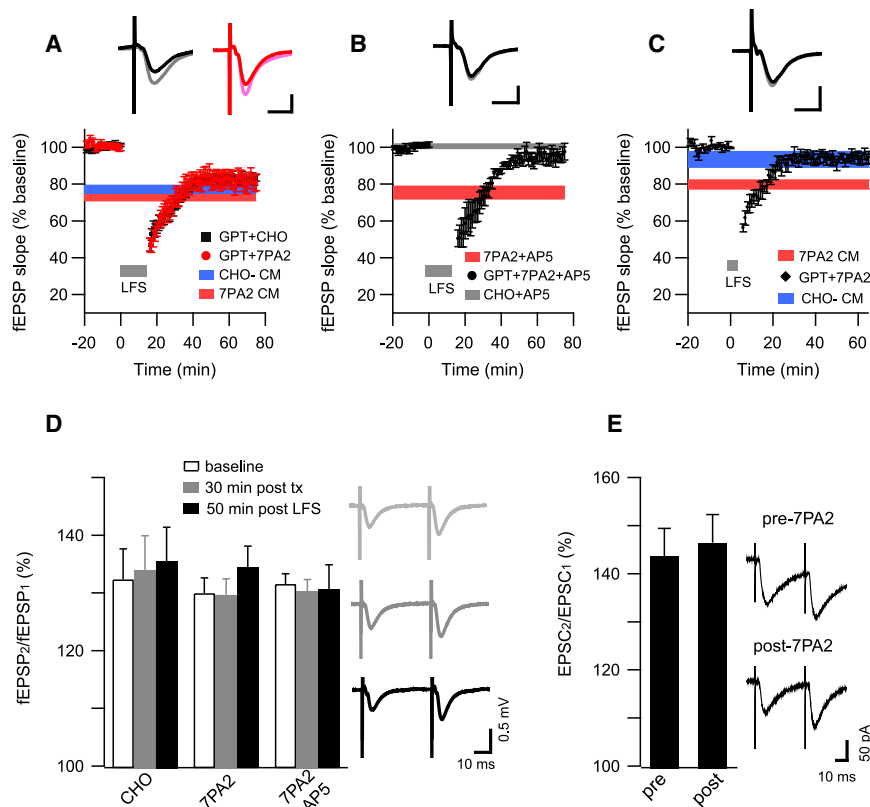


Figure 3. Selective Metabolism of Extracellular Glutamate Prevents Soluble A β -Facilitated LTD

(A) A glutamate scavenger system (glutamic pyruvic transaminase [GPT, 5 unit/ml] + pyruvate [2 mM]) has no significant effect on the fEPSP baseline and 900-pulse LTD in CHO- or 7PA2 CM treated slices. Horizontal colored bars represent the corresponding means \pm SEMs from Figure 1C. Insets in (A)–(C) represent typical fEPSPs recorded before (light) and 50 min after (dark) LFS; horizontal calibration bar, 10 ms; vertical bar, 0.5 mV.

(B) 900-pulse LTD (gray bar) was blocked by AP5 (50 μ M) in slices exposed to GPT + pyruvate for 15 min prior to 7PA2 CM ($n = 6$). Red horizontal bar shows the LTD resistant to the same dose of AP5 in the absence of exposure to scavengers. (C) 300-pulse LTD (gray bar) was significantly prevented by coadministering glutamate scavengers with the 7PA2 CM. Horizontal colored bars represent the corresponding means \pm SEMs from Figure 1B.

(D) Paired-pulse facilitation in slices exposed to 7PA2 CM, CHO- CM, or 7PA2 CM + AP5 (50 μ M) measured before (white) or 30 min after (gray) adding these media or else measured 50 min after (black) a 900-pulse induction of LTD. At right are typical traces of these field recordings from the 7PA2 group.

(E) Similar paired-pulse facilitation recorded by whole-cell voltage clamping at -70 mV in CA1 pyramidal cells before and 30 min after 7PA2 CM exposure. At right are typical traces. Data are means \pm SEMs.

7PA2 CM was not prevented by MCPG ($78.5\% \pm 4.1\%$, $n = 6$, versus $73.6\% \pm 2.6\%$, $n = 9$; $p > 0.05$) (Figure 2B). Interestingly, 50 μ M AP5 fully blocked 900-pulse LTD in slices exposed to CHO- CM but had no significant effect on the 900-pulse LTD in slices exposed to the A β -rich 7PA2 CM ($100.6\% \pm 1.4\%$, $n = 8$, versus $75.4\% \pm 3.4\%$, $n = 6$; $p < 0.01$) (Figure 2C). This AP5 resistance noted with 7PA2 CM was also observed with soluble synthetic A β_{1-42} (see Figure S1A available online) and soluble extracts of AD cortex containing A β oligomers (Supp. Figure 1B; data quantified in Supp. Figure 1C). Furthermore, application of SEC fractions of 7PA2 CM revealed that the induction of AP5-resistant 900-pulse LTD was specific to A β oligomers, not monomers (Figures 2D and S1C). These results using three distinct sources of soluble low- n oligomers suggest that the LTD facilitated by A β oligomers is mechanistically different from conventional NMDAR-dependent LTD that is sensitive to 50 μ M AP5. Because the 900-LFS protocol for inducing LTD in the CA1 region is well known to be NMDAR dependent (see Kemp and Bashir, 2001, and all control data in Figures 2C, 2D, and S1C), the resistance to AP5 of the LTD induced in the presence of the various soluble A β preparations was unexpected. It has been reported that synthetic A β can increase NMDAR activity by acting as an agonist or coagonist of NMDARs (Cowburn et al., 1997) and increasing NMDA conductance (Wu et al., 1995). Consequently, 50 μ M AP5 may not have been sufficient to completely block an enhanced NMDAR activation mediated by A β oligomers. When we used a more potent isoform of AP5, D-AP5, and increased

the concentration to 100 μ M, the soluble A β -facilitated LTD was now significantly prevented ($96.5\% \pm 3.4\%$, $n = 5$) (Figure 2E). Indeed, the block of A β -facilitated LTD was dependent on AP5 dose (Figure 2F). Rescuing LTD expression in the presence of 7PA2 CM required much higher AP5 concentrations than those needed to rescue LTD in the presence of CHO- CM (IC_{50} : 17.4 versus 72.5 μ M; $p < 0.001$), supporting the conclusion that active NMDARs are involved in LTD facilitation by soluble A β .

An Extracellular Glutamate Scavenger Restores Soluble A β -Enhanced LTD to Normal Levels

Because A β oligomers enhanced LTD through activation of glutamate receptors, we asked whether this activity of soluble A β was dependent on extracellular glutamate concentration. To this end, we used an enzymatic glutamate scavenger system (glutamic-pyruvic transaminase [GPT] + pyruvate) to reduce extracellular glutamate levels (Overstreet et al., 1997; Min et al., 1998). Exposure of hippocampal slices to GPT alone (5 units/ml) had no effect on baseline synaptic activity (data not shown). GPT + pyruvate (2 mM) treatment 15 min prior to the application of CHO- CM or 7PA2 CM affected neither baseline activity nor the magnitude of LTD induced by 900-LFS (CHO-: $82.6\% \pm 2.1\%$, $n = 5$; 7PA2: $81.8\% \pm 4.5\%$, $n = 5$) (Figure 3A); however, the LTD induced in the presence of A β and GPT could now be blocked by 50 μ M AP5 ($95.1\% \pm 3.6\%$, $n = 5$) (Figure 3B). This result suggests that elevated extracellular glutamate concentrations contribute to the enhancement of LTD by soluble A β . Similarly, treatment

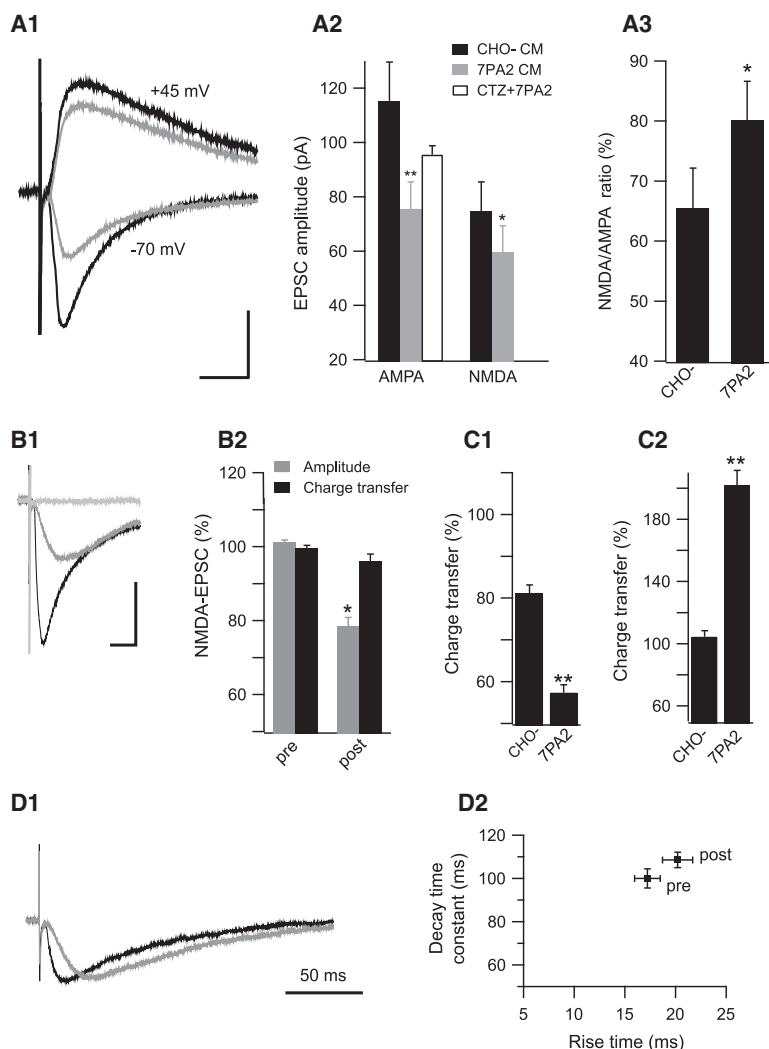


Figure 4. Soluble A β Oligomers Alter NMDAR-Mediated EPSCs in Whole-Cell Voltage-Clamp Recordings

(A1) Isolated AMPA-EPSC and NMDA-EPSC were recorded from -70 mV and $+45$ mV holding potentials, respectively, plus pharmacological blockers. Black traces, CHO-CM treated cells; gray traces, 7PA2 CM treated cells. (A2) Summary data for CHO-CM (black) and 7PA2 CM (gray) groups and for pretreatment with cyclothiazide (CTZ, $100 \mu\text{M}$) prior to 7PA2 CM (white). (A3) NMDA/AMPA EPSC ratios are different in CHO-CM and 7PA2 CM.

(B1) Typical traces from CA1 pyramidal cells held at -70 mV (black), isolated NMDA-EPSC in low Mg^{2+} with NBQX ($10 \mu\text{M}$) and bicuculline ($20 \mu\text{M}$) at -70 mV (gray), and full blockage of the NMDA current by AP5 ($50 \mu\text{M}$) (light gray). (B2) Summary data for the isolated NMDA-EPSC before (pre) and 20–30 min after 7PA2 CM exposure. Peak amplitudes (gray) and total charge transfers (black) are expressed as means \pm SEMs.

(C1) The selective NR2B inhibitor, ifenprodil ($3 \mu\text{M}$), modestly reduces NMDA charge transfers in control but markedly reduces it in 7PA2 CM. (C2) 7PA2 CM significantly increases the extrasynaptic response when synaptic NMDAR are first blocked by MK-801.

(D) NMDA-mediated EPSC kinetic analysis: (D1) representative scaled traces before (black) versus after (gray) 7PA2 CM exposure; (D2) summary data of rise time plotted versus decay time before (pre) and after (post) 7PA2 CM exposure. Data are means \pm SEMs.

The A β -Mediated Increase in Synaptic Glutamate Concentration Alters Properties of Excitatory Transmission

We next sought to determine more precisely the basis of soluble A β -mediated alterations in glutamatergic transmission through whole-cell recordings. AMPAR-mediated currents (AMPA-EPSC) and NMDAR-mediated currents (NMDA-EPSC) were recorded from pyramidal neurons in slices treated with CHO-CM or 7PA2 CM (Figure 4A1). Both

currents were significantly reduced by the soluble A β -rich 7PA2 CM compared to the CHO-CM (AMPA $67\% \pm 4\%$, $n = 18$, $p < 0.001$; NMDA $79\% \pm 3\%$, $n = 13$, $p < 0.01$) (Figure 4A2). This result was unexpected, as the increased extracellular glutamate concentrations observed above should increase AMPA and/or NMDA currents. However, the result could be explained if the A β -mediated rise in glutamate levels leads to receptor desensitization. To address this possibility, we applied a well-characterized inhibitor of AMPAR desensitization, cyclothiazide (CTZ, $100 \mu\text{M}$), to the slices before exposure to 7PA2 CM; the AMPA EPSC was no longer significantly decreased (CHO-CM: 115 ± 14 pA versus 7PA2 CM + CTZ: 96 ± 3 pA, $n = 10$; $p > 0.05$) (Figure 4A2). Interestingly, we observed spiking activity escaping voltage clamp in 6/10 cells recorded in the 7PA2 CM + CTZ condition (Figures S2A and S2B) but not in the CTZ-only condition (three cells; data not shown), further suggesting alterations in excitatory transmission due to supraphysiologic levels of extracellular glutamate in the presence of soluble A β . To assess whether AMPAR desensitization is involved in the induction of LTD by soluble A β , CTZ was applied to brain slices, and 900-pulse

with the GPT + pyruvate also prevented the 300-LFS A β -induced LTD ($94.4\% \pm 2.8\%$, $n = 5$) (Figure 3C). We conclude that the enhancement of LTD by soluble A β in both mGluR-dependent and NMDAR-dependent conditions involves elevated extracellular glutamate levels.

Paired-pulse facilitation (PPF) reveals whether alterations of synaptic transmission are presynaptic. To assess whether soluble A β oligomers increase extracellular glutamate levels by affecting presynaptic release probability, we measured PPF in slices exposed to CHO-CM, 7PA2 CM, or 7PA2 CM + AP5 ($50 \mu\text{M}$) at baseline, 30 min after these exposures, and then 50 min after a 900-pulse LTD induction. PPF was not significantly different between these three conditions (Figure 3D). To establish further that soluble A β does not alter presynaptic release, we performed whole-cell recordings of pyramidal cells and found that PPF ratios before and 30 min after 7PA2 CM exposure were not significantly different (Figure 3E). The experiments described so far suggest that the facilitation of LTD by soluble oligomeric A β involves elevated levels of extracellular glutamate, but not by altering presynaptic release probability.

LTD was induced; the LTDs were not significantly different in CHO- versus 7PA2 CM (Figure S2C), suggesting that AMPAR desensitization is not required for the LTD induction.

To preserve the neuronal glutamate uptake expected under normal physiological function, we repeated the single-cell recordings at -70 mV rather than at positive potentials (Grewer and Rauen, 2005). NMDA-EPSC was isolated in low Mg^{2+} (0.1 mM) and with pharmacological blockade of AMPAR (with NBQX, 10 μ M) and GABA-R (with BIC, 20 μ M) (Figure 4B1). Under these conditions, the NMDAR-EPSC was again found to be decreased by 7PA2 CM ($78\% \pm 3\%$, $n = 10$; $p < 0.01$) (Figure 4B2). The NMDA-EPSC reduction could be due to NMDAR desensitization in the presence of increased extracellular glutamate levels (Sarantis et al., 1993). To better understand the NMDA current changes, we quantified charge transfers, calculated as the area under the curve of the NMDA-EPSC. In contrast to the reduction in peak amplitude, the total charge transfer was not decreased by the A β -rich 7PA2 CM ($96\% \pm 2\%$ of pretreatment values, $n = 11$; $p > 0.05$) (Figure 4B2). Because NR2B receptors have a 2- to 3-fold higher sensitivity for glutamate than do NR2A receptors (Kutsuwada et al., 1992), we asked whether NR2B receptors played the principal role in the A β -mediated effect on NMDA currents. The selective NR2B receptor inhibitor, ifenprodil (3 μ M), modestly inhibited the NMDA-EPSC total charge transfer under control (CHO- CM) conditions ($81\% \pm 2\%$, $n = 8$) (Figure 4C1). However, ifenprodil strongly and significantly inhibited the NMDA-EPSC charge transfer in the presence of 7PA2 CM ($55\% \pm 2\%$, $n = 9$; $p < 0.05$) (Figure 4C1), suggesting enhanced activation of NR2B-subunit-containing NMDA receptors. The NR2B subunit is considered to be predominantly present in extrasynaptic NMDA receptors (Tovar and Westbrook, 1999). We selectively blocked synaptic NMDAR with MK-801, an irreversible, use-dependent NMDA channel blocker, washed it out, and perfused with 7PA2 CM under the above NMDA-EPSC isolating conditions. The NMDA-EPSCs were markedly increased ($202\% \pm 10\%$ of the CHO- CM level, $n = 5$; $p < 0.01$) (Figure 4C2). Taken together, the above results suggest that soluble A β oligomers increase NR2B-mediated NMDAR activity and enhance extrasynaptic responses.

Spillover of increased extracellular glutamate remained as the most parsimonious explanation for the relative increases in NMDA currents, activation of NR2B receptors, and extrasynaptic responses in the presence of A β oligomers. To further support this interpretation, we analyzed the NMDA EPSC kinetics, i.e., the 10%–90% rise time and the decay time constant. While the peak amplitude decreased slightly after 7PA2 CM treatment, the rise time (16 ms versus 20 ms for pre- versus postexposure; $p < 0.05$) and decay time (100 ms versus 109 ms for pre- versus postexposure; $p < 0.05$) were significantly prolonged (Figure 4D), suggesting increased diffusion distance and dwelling times, i.e., that neurotransmitter spillover is enhanced upon exposure to soluble A β oligomers.

Soluble A β Enhances LTD through Inhibition of Glutamate Uptake

Our findings above that soluble A β -enhanced LTD may activate a larger population of NMDARs can be considered in terms of A β interrupting glutamate reuptake (Harkany et al., 2000; Gu et al., 2004). To investigate this possibility, we examined the

effects of a well-characterized glutamate uptake inhibitor, TBOA. Soluble A β extracted from AD brain or present in 7PA2 CM (Figure 1B) facilitates LTD in CA1 after a 300-LFS protocol, whereas this low stimulus induces weak or no LTD in control-treated slices. To test whether inhibiting glutamate uptake itself facilitates LTD, the 300-LFS protocol was applied after a 30 min TBOA treatment (15 μ M). Closely similar to our results with soluble A β treatment in this 300-LFS mode (Figure 1B), we found that TBOA alone induced LTD in an NMDAR-independent but mGluR-dependent manner (TBOA alone: $74\% \pm 4.5\%$, $n = 5$; TBOA + AP5: $78.1\% \pm 3.5\%$, $n = 5$; TBOA + MCPG: $91\% \pm 4.6\%$, $n = 6$) (Figure 5A). Similar to the 900-pulse LTD induced in the presence of soluble A β (Figure 2), the 900-pulse LTD induced in the presence of TBOA was also resistant to 50 μ M AP5 but was prevented by 100 μ M D-AP5 (TBOA: $73.5\% \pm 3.4\%$, $n = 5$; TBOA + AP5: $76.9\% \pm 5.6\%$, $n = 5$; TBOA + D-AP5: $94.2\% \pm 2.8\%$, $n = 5$; $p < 0.01$ compared to TBOA + AP5) (Figure 5B).

To assess whether TBOA-enhanced LTD has mechanistic similarity with soluble A β , we recorded the TBOA-induced LTD in a repetitive 900-LFS paradigm to saturate the LTD response, then washed out the TBOA and replaced it with 7PA2 CM (Figure 5C). When 900-LFS was then performed, there was no further increase in LTD ($96.1\% \pm 9.1\%$ of renormalized baseline measured 40 min after LFS, $n = 5$; $p > 0.05$) (Figures 5C and 5D). This occlusion experiment suggests that these two forms of LTD share similar mechanisms. Interestingly, if we first applied 7PA2 CM and saturated the LTD, then the usual dose (15 μ M) of TBOA significantly reduced baseline neurotransmission (to 78% of the renormalized baseline at 30 min) (Figure S3A), and this effect was similar to that of a higher dose of TBOA alone (50 μ M) on the baseline (81% at 30 min) (Figure S3B). The latter results further substantiate a shared mechanism between TBOA and soluble A β on perturbation of neurotransmission. We also examined DHK (500 μ M), an inhibitor of glial glutamate transporter (GLT-1), but the 900-pulse LTD observed in the presence of this agent was largely prevented by the standard 50 μ M AP5 dose ($89.2\% \pm 3.2\%$, $n = 6$, versus $77.5\% \pm 2.9\%$, $n = 6$, $p < 0.05$, Figure S3C), suggesting specificity for neuronal, not glial, glutamate transporters in the enhancement of the 900-pulse LTD.

The effects of soluble A β in inhibiting HFS-induced LTP have been well established using several types of A β preparations, and we confirmed this here using soluble A β -rich 7PA2 CM (CHO- CM: $144.7\% \pm 5.4\%$, $n = 7$, versus 7PA2 CM: $108.1\% \pm 6.6\%$, $n = 7$; $p < 0.001$) (Figure 5E). TBOA similarly inhibited HFS-induced LTP (vehicle: $151.3\% \pm 4.8\%$, $n = 6$, versus TBOA: $112.2\% \pm 3.1\%$, $n = 6$; $p < 0.001$) (Figure 5F). These results support our conclusion that the effects of soluble A β on synaptic plasticity mimic those observed with the blockade of neuronal glutamate uptake.

To assess directly whether soluble A β inhibits glutamate uptake as all of the above data suggest, we measured the uptake of radiolabeled glutamate by isolated synaptosomes (see Experimental Procedures). As a positive control, TBOA decreased glutamate uptake in the synaptosomal preparation in a dose-dependent manner ($IC_{50} = 73.9$ μ M) (Figure 5G). Synthetic A β_{1-42} significantly decreased glutamate uptake into synaptosomes ($69\% \pm 6\%$ of vehicle treatment, $n = 15$; $p < 0.01$), similar to the effects of TBOA ($41\% \pm 4\%$ at 50 μ M, $n = 5$; $p < 0.01$)

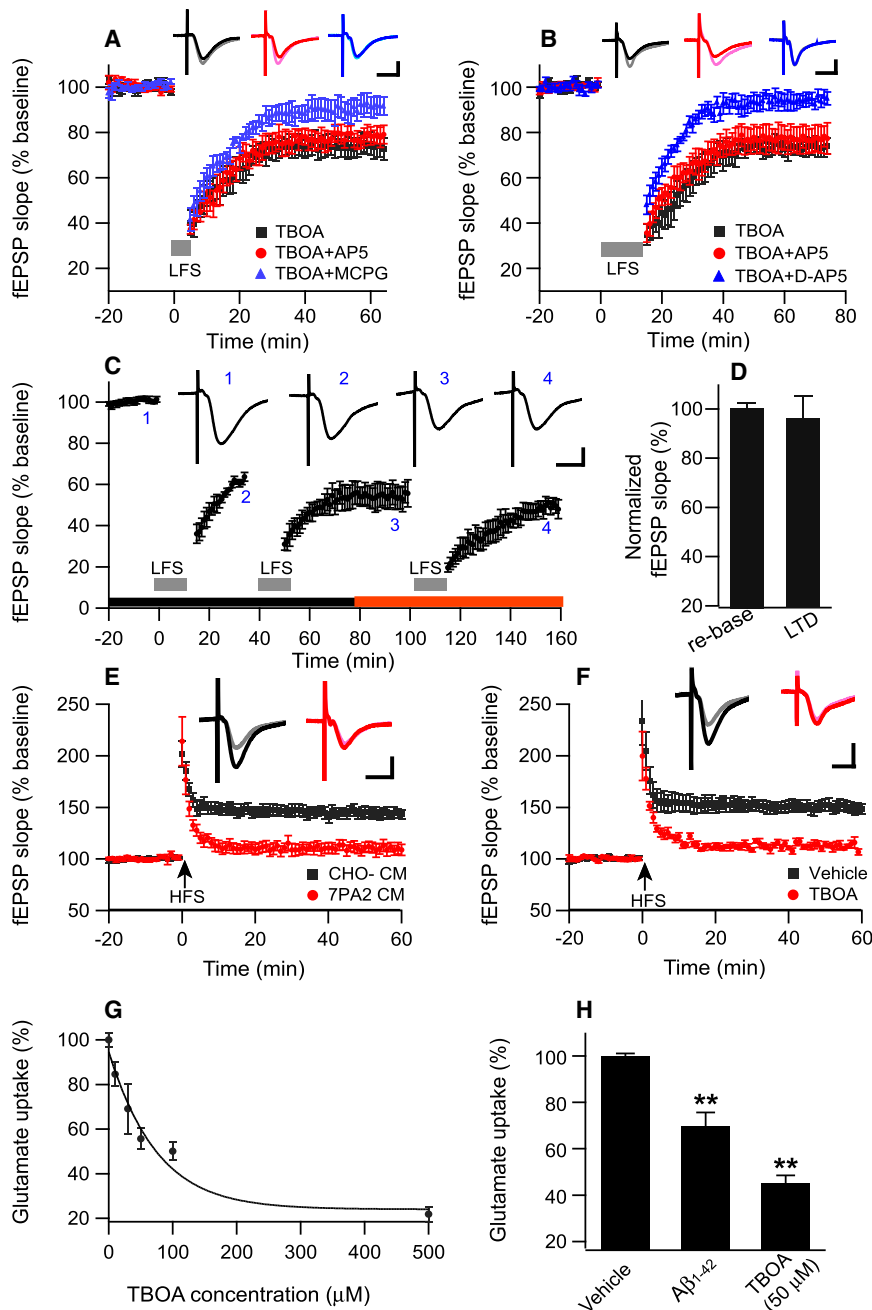


Figure 5. Soluble A β -Enhanced LTD Involves Impaired Glutamate Reuptake

(A) LTD was induced by 300 pulses (gray bar) when just the glutamate uptake inhibitor, TBOA (15 μ M), was perfused (black squares, $n = 5$). This TBOA-mediated LTD was prevented by mGluR antagonist MCPG (500 μ M, blue triangles, $n = 6$) but not by NMDAR antagonist AP5 (50 μ M, red circles, $n = 6$).

Insets in (A)–(F) represent fEPSPs recorded before (light) and 50 min after (dark) either LFS (in A–C) or HFS (in E and F). Horizontal calibration bars, 10 ms; vertical bars, 0.5 mV.

(B) A 900-pulse LTD facilitated by TBOA was resistant to a standard dose of AP5 (50 μ M, red circles, $n = 6$), but it could be blocked by D-AP5 (100 μ M, blue triangles, $n = 6$).

(C) Saturation of 900-pulse LTD occurring in the presence of TBOA (black horizontal bar) occludes further LTD in the presence of 7PA2 CM (red horizontal bar).

(D) Summary data from occlusion experiments as in (C): left bar, evoked fEPSP was renormalized to baseline values (i.e., point 3 in C) before addition of 7PA2 CM to the perfusate and an additional LFS (right bar).

(E) LTP induced by high-frequency stimulation (HFS) was prevented in slices in 7PA2 CM (red circles) but unaffected in slices in CHO-CM (black squares).

(F) HFS-induced LTP was similarly prevented by TBOA.

(G) Dose-dependent inhibition of glutamate uptake by TBOA in hippocampal synaptosomes.

(H) Pretreatment with soluble synthetic A β 1-42 impaired glutamate uptake in hippocampal synaptosomes in a fashion similar to TBOA treatment at 50 μ M. Data are means \pm SEMs as percentage of vehicle alone; ** $p < 0.01$.

(Figure 5H). This biochemical evidence further supports our hypothesis that soluble A β impairs neuronal glutamate uptake and can thereby increase extracellular glutamate concentration and subsequent NMDAR activation.

Extracellular Calcium Influx, Not Intracellular Stores, Contributes to Soluble A β -Facilitated LTD

To assess whether alterations in calcium influx through NMDARs mediate the facilitation of LTD by soluble A β , the ACSF calcium concentration was reduced to 0.5 mM prior to the application of soluble A β and 900-LFS to the brain slices. This reduction of extracellular calcium concentration prevented LTD induction in

the presence of either 7PA2 or CHO-CM (Figure 6A). To test whether extracellular calcium is required for the NMDAR-mediated enhancement of LTD by soluble A β , we applied 50 μ M AP5 and varied the extracellular Ca $^{2+}$ concentration between 0.5 mM and 6 mM (Figure 6A). The LTD induced by 900-LFS in the presence of AP5 + CHO-CM was fully blocked when the Ca $^{2+}$ concentration was 1 mM or 2.5 mM, and only a small AP5-resistant LTD ($86.4\% \pm 4.5\%$, $n = 6$) occurred at the highest Ca $^{2+}$ concentration (6 mM). In contrast, LTD induced in presence of 7PA2 CM was significantly prevented by 50 μ M AP5 at low (1 mM) Ca $^{2+}$ concentration (7PA2 CM: $80\% \pm 4.2\%$, $n = 5$, versus 7PA2 CM + AP5: $93.2\% \pm 3.4\%$, $n = 6$; $p < 0.05$) but not at normal (2.5 mM: $75.4\% \pm 3.4\%$, $n = 6$) or high (6 mM: $70.4\% \pm 3.4\%$, $n = 5$) Ca $^{2+}$ concentrations. These results indicate that A β -enhanced LTD is dependent on extracellular calcium concentration.

To assess whether intracellular calcium stores also regulate A β -mediated LTD, as they have been reported to do for A β neurotoxicity (Demuro et al., 2005), we tested two compounds that

inhibit the release of intracellular Ca²⁺ stores, ryanodine and U73122. Although ryanodine (20 μ M) and U73122 (10 μ M) each prevented 900-LFS LTD induction in slices treated with CHO-CM, they did not alter the LTD facilitation by 7PA2 CM (87% \pm 2.7%, n = 5, versus 76% \pm 3.2%, n = 5, for ryanodine, p < 0.05) (Figure 6B) (96.2% \pm 3.4%, n = 4, versus 74.6% \pm 4.6%, n = 6, for U73122, p < 0.01) (Figure 6C). This suggests that soluble A β -facilitated LTD requires extracellular calcium influx but not intracellular calcium release.

Activation of Calcineurin and GSK-3, but Not p38 MAPK, Is Required for A β -Mediated LTD

When cytosolic calcium reaches critical concentrations, certain LTD-related signaling pathways are activated (Kemp and Bashir, 2001). For example, NMDAR-dependent LTD in the CA1 region recruits calcineurin (PP2B) and p38 MAPK cascades (Mulkey et al., 1994; Bolshakov et al., 2000; Li et al., 2006). We examined these two signaling pathways using their respective inhibitors, FK506 (20 μ M) and SB203580 (5 μ M). Whereas blocking p38 MAPK activation with SB203580 prevented conventional 900-pulse LTD induction (i.e., in CHO-CM), it did not significantly affect the LTD induced in the presence of soluble A β -rich 7PA2 CM (94.6% \pm 2.5%, n = 7, versus 73.9% \pm 4%, n = 6; p < 0.01) (Figure 6D). In contrast, PP2B function was required for LTD induced both in the absence and presence of soluble A β (Figure 6E). Glycogen synthase kinase-3 (GSK3)-mediated signaling was recently reported to play an important role in LFS-induced LTD (Peineau et al., 2007), and it has also been implicated in A β -mediated neurotoxicity in cultured hippocampal slices (Nassif et al., 2007). Accordingly, we treated slices with the GSK-3 β inhibitor, SB415286 (10 μ M), for 30 min prior to applying either CHO-CM or 7PA2 CM and then performed 900-LFS. LTD could not be induced in either condition (94.3% \pm 4.7%, n = 6, versus 98.4% \pm 2.6%, n = 6) (Figure 6F), suggesting that GSK-3 β activity is required for both conventional and soluble A β -mediated LTD. To further verify that glutamate uptake inhibition shares similar mechanisms with soluble A β as regards LTD induction, ryanodine, SB203580, and FK506 were each applied to the slices prior to treatment with TBOA. Entirely consistent with the results for A β -mediated LTD, both intracellular Ca²⁺ release and p38 MAPK activation were not required for the TBOA-mediated LTD, whereas PP2B activity was (Figures 6G–6I).

To provide further information about the receptor mechanisms of LTD facilitation by soluble A β , we took advantage of Ras-guanine nucleotide-releasing factor 1 (Ras-GRF1) knockout mice, which are deficient in the ability to mount an LTD response (Li et al., 2006). Ras-GRF proteins are best known for their ability to activate Ras/Erk through their CDC25-GEF domains and activate the Rac/p38 cascade through their DH-GEF domains, which act as calcium sensors for different classes of NMDARs and drive the activation of distinct MAP kinase family members. As expected, hippocampal slices from these *Ras-GRF1*^{−/−} mice did not express LTD when 900-LFS was administered in the presence of CHO-CM. In contrast, LTD was elicited in the presence of the oligomer-rich 7PA2 CM (99.4% \pm 2.9%, n = 6, versus 75.1% \pm 2.9%, n = 7; p < 0.01) (Figure S4A). This soluble A β -facilitated LTD in the *Ras-GRF1*^{−/−} mice was not blocked by 500 μ M MCPG (Figure S4B) nor by 50 μ M AP5 (Figure S4C), but it was

significantly decreased by 100 μ M AP5 (91.6% \pm 3.1%, n = 5, p < 0.05) (Figure S4C). Furthermore, the p38 MAPK inhibitor (SB203580) had no effect on the A β -facilitated LTD (80.2% \pm 4.8%, n = 5) (data not shown), whereas pretreatment with either the calcineurin inhibitor (FK506) or the GSK-3 β inhibitor (SB415286) significantly prevented the LTD associated with 7PA2 CM (95.6% \pm 4.8%, n = 6, and 88.4% \pm 4.7%, n = 6, respectively) (Figure S4D). Therefore, soluble A β bypassed the nonfunctional LTD induction pathway in *Ras-GRF1*^{−/−} mice, a pathway that is normally sensitive to 50 μ M AP5 in wild-type mice.

DISCUSSION

Given the mounting evidence that soluble A β oligomers mediate synaptic impairment in AD, elucidating the precise molecular pathways by which this occurs has important implications for treating and preventing the disease. Here, we demonstrate that soluble A β oligomers facilitate LTD in the hippocampus through a mechanism that appears to involve the inhibition of glutamate uptake. With a weak (300-pulse) LFS, A β -mediated LTD was prevented by MCPG but not AP5, and with a conventional (900-pulse) LFS, the LTD was prevented only by a high (100 μ M), not a standard (50 μ M), dose of AP5. Extracellular glutamate scavengers effectively prevented A β -enhanced LTD. Mechanistically, soluble A β oligomers caused glutamate spillover, and their ability to facilitate LTD required an influx of extracellular calcium but did not detectably require Ca²⁺ release from intracellular stores. Once Ca²⁺ enters the neuron, it is known to trigger PP2B and GSK3 signaling pathways, and we implicated these in the A β -facilitated LTD induction.

Importantly, A β facilitation of LTD was closely mimicked by pharmacologically inhibiting neuronal (but not glial) glutamate uptake, consistent with the rescue of A β -LTD by the glutamate scavenger. In accord, we found that A β significantly impaired glutamate uptake by synaptosomes, suggesting that A β facilitates LTD in part by altering synaptic glutamate recycling. These results support the hypothesis that excess synaptic glutamate caused by A β mechanistically alters LTD induction in hippocampus. It should be emphasized that the effects of soluble A β we describe in this study are attributable to soluble oligomers (Figure 1A), as no larger assemblies (protofibrils, fibrils) were present, and SEC-isolated oligomers but not monomers conferred the effects on LTD (Figure 2D). A summary mechanism incorporating all of our findings is proposed in Figure 7.

We focused on the NMDAR-dependent induction of LTD, a widely used protocol for LTD studies. There have been contradictory findings among the few studies of the effects of A β on LTD (Kim et al., 2001; Wang et al., 2002; Raymond et al., 2003; Wang et al., 2004a). Hsieh et al. (2006) showed that A β secreted by neurons in hippocampal slices transfected with mutant human APP could induce LTD via an mGluR-dependent mechanism involving AMPAR internalization. Moreover, Chang et al. (2006) reported that aged APP/Presenilin-1 double knockin mice having reduced AMPAR mEPSCs were resistant to LTD induction, suggesting a floor effect for synapse depression following prolonged exposure to A β . Our new data indicate that soluble A β oligomers can induce hippocampal LTD, although the mechanisms do not follow canonical p38 MAPK pathways.

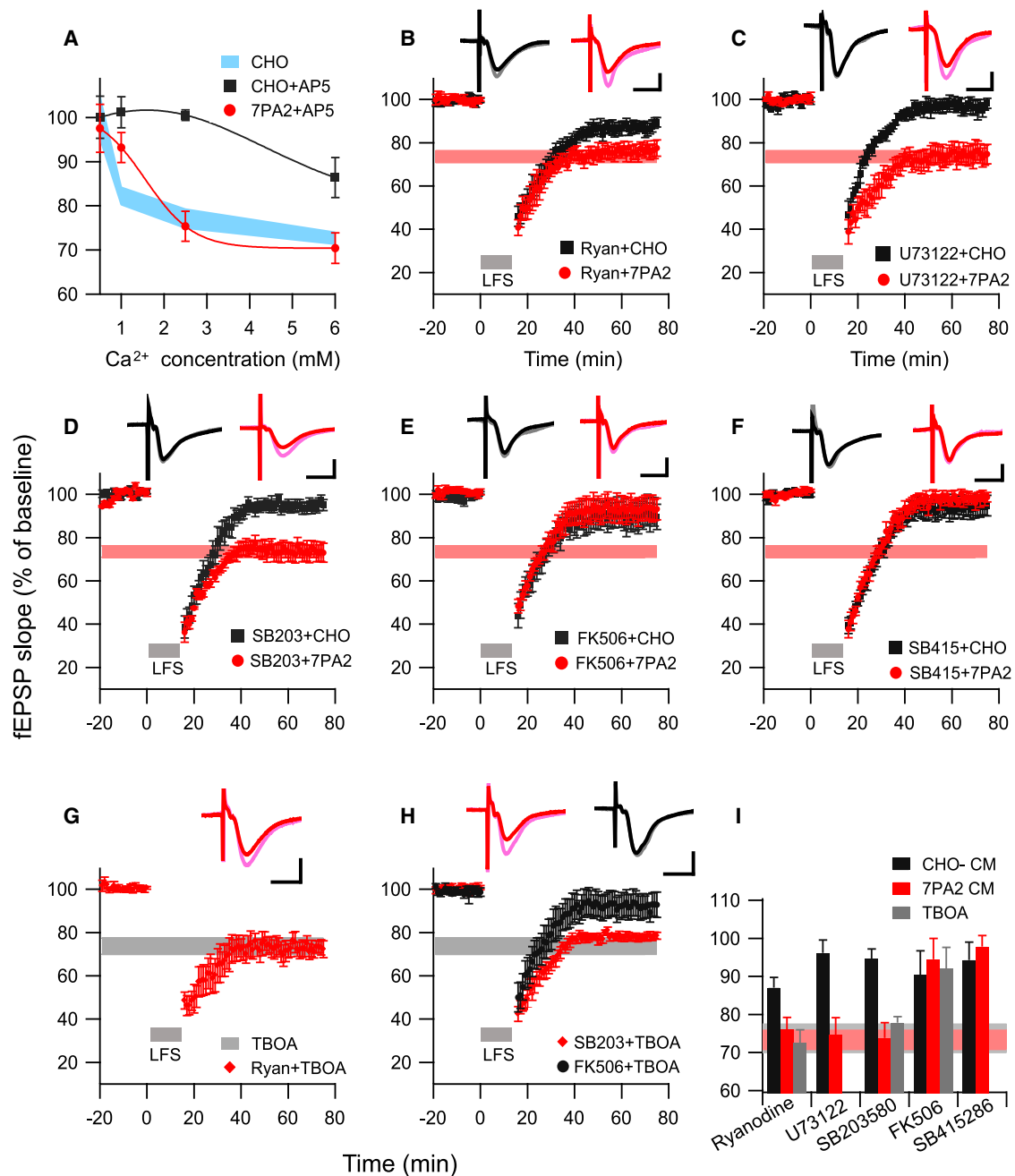


Figure 6. LTD Facilitated by Soluble A β or TBOA Share Similar Signaling Pathways

(A) Conventional LTD (in CHO- CM, black squares) and A β -enhanced LTD (in 7PA2 CM, red circles) induced by the 900-pulse LFS in the presence of AP5 (50 μ M) were plotted as a function of increasing extracellular calcium concentrations in the perfusate. Light blue area represents the LTD obtained in the CHO- CM alone (without AP5). Data are means \pm SEMs.

(B) Ryanodine (20 μ M) given 30 min prior to 900 pulses partially blocked the LTD in CHO CM (black squares, $n = 5$) but produced no block of the 7PA2-facilitated LTD (red circles, $n = 6$).

(C) U73122 (10 μ M) (applied 45 min prior to 900 pulses) fully blocked LTD in the presence of CHO- CM (black squares, $n = 6$) but had no effect in the presence of 7PA2 CM (red circles, $n = 6$).

(D) 900-pulse LTD was blocked by p38 MAPK inhibitor, SB203580 (5 μ M), in CHO- CM (black squares, $n = 7$) but not in 7PA2 CM (red circles, $n = 7$).

(E) Calcineurin inhibitor, FK-506 (20 μ M) perfused 40 min prior to 900 pulses prevented LTD in both CHO- CM (black squares, $n = 5$) and 7PA2 CM (red circles, $n = 6$).

(F) GSK-3 β inhibitor, SB415286 (10 μ M) perfused 60 min prior to 900 pulses prevented LTD in both CHO- CM (black squares, $n = 6$) and 7PA2 CM (red circles, $n = 7$).

(G) LTD enhanced by TBOA (15 μ M) was likewise resistant to ryanodine ($n = 5$).

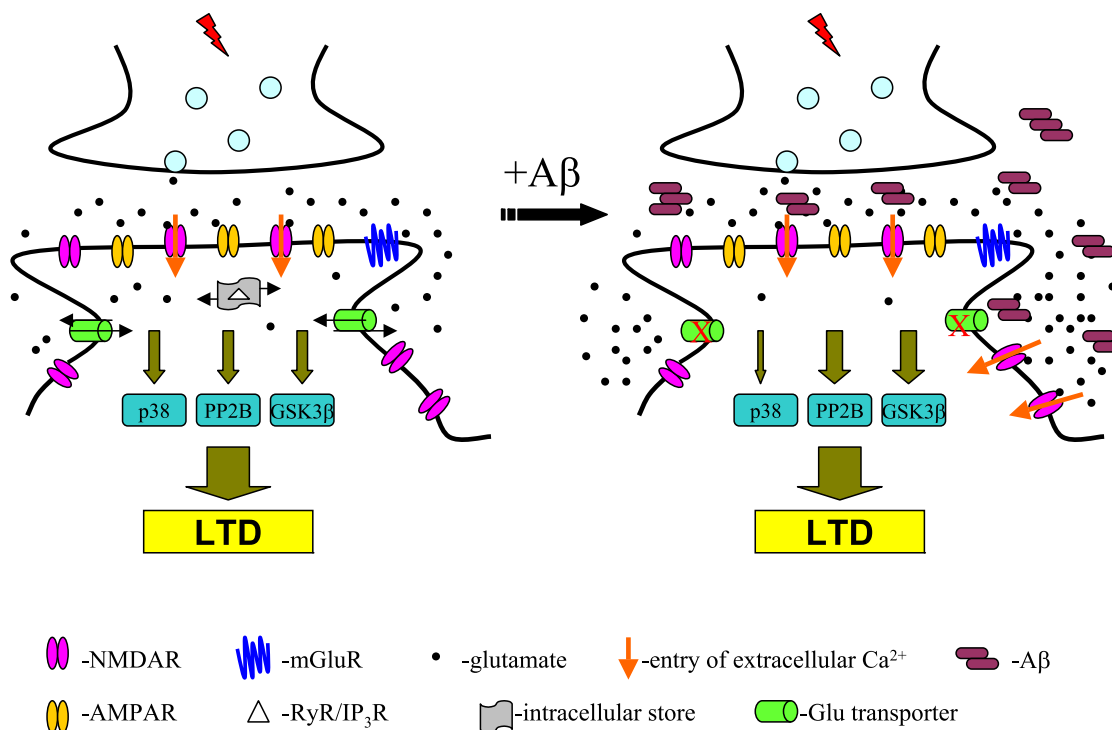


Figure 7. Schematic of the Principal Pathways Implicated by This Study in Conventional LTD and in LTD Facilitated by Soluble A β Oligomers
(Left panel) Conventional LTD requires NMDAR-mediated influx of extracellular calcium and liberation of intracellular calcium stores. This ultimately activates PP2B, GSK-3 β , or p38 MAPK signaling pathways that induce LTD. (Right panel) Soluble A β oligomers lead to activation of more NMDAR, leading to extracellular calcium influx and activation of PP2B and GSK-3 β pathways to facilitate LTD. Our data suggest that A β oligomers decrease glutamate uptake by neuronal transporters (red x's), resulting in the enhanced activation of NMDARs and thus facilitation of LTD-inducing pathways.

Synthetic A β has been shown to increase NMDAR-mediated currents in rat dentate gyrus (Wu et al., 1995) or enhance NMDA response in the hippocampus after a 15 min exposure in vivo (Molnár et al., 2004). Our current results show that soluble A β oligomers depressed AMPAR currents (Figures 4A and 4B), but this was likely due to desensitization. Earlier work has suggested that NR2B-containing NMDARs play a major role in normal LTD induction (Liu et al., 2004), although some recent evidence is not in agreement (Morishita et al., 2007). Our study suggests that soluble A β oligomers can significantly increase NR2B receptor activation, as demonstrated by an increased ifenprodil-sensitive component of the NMDAR-EPSC following exposure to soluble A β . NR2B receptors are specifically coupled to SynGAP (Kim et al., 2005), and their activation would thus inhibit the Ras-Erk signaling pathway known to be required for LTP, instead favoring LTD induction. Although some studies have colocalized A β with NMDAR at synapses (Dewachter et al., 2009; Lacor et al., 2007), it remains unclear whether these are a direct target of the peptide, and other studies have suggested A β interactions with voltage-dependent calcium channels (VDCCs) (Ueda et al., 1997), mGluRs (Wang et al., 2004b),

nicotinic receptors (Dineley et al., 2001; Snyder et al., 2005), or insulin receptors (Townsend et al., 2007), all of which could modulate NMDAR activity. It remains plausible that the hydrophobic A β oligomers actually interact with lipid and/or protein targets upstream of these various receptors, perturbing their function secondarily.

Extrasynaptic glutamate spillover is a phenomenon that has been implicated in cross-talk among hippocampal synapses (Asztely et al., 1997; Min et al., 1998; Scimemi et al., 2004). Inhibition of glutamate uptake at the synapse (e.g., by soluble A β) can significantly increase the extracellular glutamate concentration and thereby enhance glutamate spillover to neighboring synapses. Electrophysiologically, NMDA currents will be increased and their rise and decay time courses significantly prolonged, due to the increased distance between the glutamate release site and the target receptors. In addition, extrasynaptic NMDAR activity will be increased by synaptic glutamate accumulation. Our present results do not fully meet these criteria, as we found that soluble A β depressed peak NMDAR currents. We hypothesize that A β oligomers depress NMDA currents as a result of desensitization of the receptors. It is of related interest that several glutamate uptake

(H) TBOA-enhanced LTD was prevented by the calcineurin inhibitor (FK 506, black circles, $n = 5$) but not by the p38 MAPK inhibitor (SB203580, red diamonds, $n = 5$).

(I) Summary data for actions of the signaling pathway modulators on A β - and TBOA-enhanced LTD. The horizontal gray bar represents mean LTD in TBOA alone (Figure 5B), and the horizontal light orange bar represents mean LTD in 7PA2 CM alone (Figure 1C). Data are means \pm SEMs.

inhibitors have been shown to depress NMDA currents (Sarantis et al., 1993; Maki et al., 1994; Asztely et al., 1997), as we show for soluble A β oligomers here. Thus, A β -mediated decreases in glutamate uptake and consequent accumulation of extracellular glutamate could induce NMDAR desensitization and/or activation of presynaptic mGluRs, thereby reducing the peak amplitudes of the NMDA currents.

Glutamate excitotoxicity has been hypothesized to have a role in AD pathogenesis. Dysfunction of glutamate transporters has been implicated in this pathway (Masliah et al., 1996; Jacob et al., 2007). Synthetic A β has been shown to inhibit glutamate uptake in cultured astrocytes (Harris et al., 1996; Harkany et al., 2000; but see Ikegaya et al., 2002) and oocytes (Gu et al., 2004). Extracellular glutamate concentration is tightly controlled in the brain by a family of membrane transporters, predominantly expressed by perisynaptic astrocytes (Danbolt, 2001). Their role is to regulate the glutamate released at synapses and to prevent spillover of the transmitter to extrasynaptic receptors. Our results showing that soluble A β facilitates LTD through both mGluR (300-LFS protocol) and NMDAR (900-LFS protocol) pathways is supported by our concomitant finding that a common upstream element, the glutamate transporter, is also regulated by soluble A β . In vivo microdialysis recently revealed that microinjection of the soluble A β -rich 7PA2 CM can significantly increase the interstitial fluid levels of glutamate—but not GABA or aspartate—in hippocampus (O'Shea et al., 2008). Inhibition of glutamate uptake by TBOA has been shown to increase spontaneous epileptiform discharges, and this increased excitability can be blocked by AP5 (Campbell and Hablitz, 2004). Interestingly, A β has also been found to significantly increase spontaneous nonconvulsive seizure activity in cortical and hippocampal networks in APP-transgenic mice (Palop et al., 2007). The precise mechanism by which soluble A β oligomers interfere with glutamate transporters at the synapse remains to be determined. Given the numerous distinct receptors and channel proteins reported to be altered by A β to date, we speculate that the hydrophobic A β oligomers bind principally to membrane lipids and thereby secondarily interrupt the structure and function of synaptic transmembrane transporters and channels, leading to changes in the transduction of intracellular signaling cascades. Such a mechanism would be consistent with the direct effect of soluble A β on glutamate uptake by isolated synaptosomes in vitro (Figure 5H).

Calcium is a second messenger that controls many cellular processes, including neuronal excitability, synaptic plasticity, and neuronal death. The increase in postsynaptic calcium that results in LTD can arise from a number of sources. For example, extracellular Ca²⁺ influx can occur through NMDARs or VDCCs (Kemp and Bashir, 2001), and intracellular Ca²⁺ release can stem from activation of the ryanodine receptor (RyR) and the inositol 1,4,5-trisphosphate receptor (IP3R). Our data (Figures 6A–6C) show that conventional hippocampal LTD requires extracellular Ca²⁺ influx through NMDARs and recruits intracellular stores from IP3R and possibly RyR, in line with previous studies on hippocampal LTD (Kemp and Bashir, 2001). However, among these possible calcium sources, A β -facilitated LTD only required NMDAR activation and enhanced extracellular calcium entry. An increase in cytosolic calcium can trigger

signaling cascades involved in LTD induction, including PP2B, p38 MAPK, and GSK3 (Mulkey et al., 1994; Bolshakov et al., 2000; Li et al., 2006; Peineau et al., 2007). The differences between our study and previous reports implicating A β in the activation of p38 MAPK (Hsieh et al., 2006; Origlia et al., 2008) could lie in the experimental conditions, for example, acute slices versus dissociated neuronal cultures or field versus whole-cell recordings.

In conclusion, the findings described here suggest that soluble A β oligomers facilitate LTD through both NMDARs and mGluRs by interfering with glutamate recycling at the synapse (Figure 7). Our study also suggests that additional mechanisms may underlie LTD in AD pathological states beyond those described previously through the NMDAR-p38/MAPK pathway. The receptors and signaling molecules activated by A β as described in this study could represent additional therapeutic targets in Alzheimer's disease.

EXPERIMENTAL PROCEDURES

A β Preparations

Secreted human A β peptides were collected and prepared from the conditioned medium (CM) of a CHO cell line (7PA2) that stably expresses human APP751 containing the V717F AD mutation (Podlisny et al., 1995). Cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum, 1% penicillin/streptomycin, 2 mM L-glutamine, and 200 mg/ml G418 for selection. Upon reaching ~95% confluency, the cells were washed and cultured overnight (~15 hr) in serum-free medium. CM was collected, spun at 1500 \times g to remove dead cells and debris, and stored at 4°C. The CM was concentrated 10-fold with a YM-3 Centricon filter (Walsh et al., 2005). Aliquots of concentrated 7PA2 CM were stored at –80°C. Synthetic A β _{1–42} (Biopolymer Lab., Dept. of Neurology, UCLA) was dissolved in water and incubated at 37°C for 10 days, then aliquoted (50 μ l) and stored at –80°C.

Hippocampal Slice Electrophysiology

The procedures for the hippocampal slice preparation and field excitatory postsynaptic potentials (fEPSPs) recordings in the CA1 region of mouse hippocampus were described previously (Li et al., 2006; Shankar et al., 2008). A bipolar stimulating electrode (FHC Inc., Bowdoin, ME) was placed in the Schaffer collaterals to deliver test and conditioning stimuli. A borosilicate glass recording electrode filled with artificial cerebrospinal fluid was positioned in stratum radiatum of CA1. Test responses were recorded for 30–60 min prior to beginning the experiment to ensure stable responses. The field potentials were amplified 100 \times using an Axon Instruments 200B amplifier and digitized with Digidata 1322A. Traces were obtained by pClamp 9.2 and analyzed using the Clampfit 9.2 program. To induce LTD, either 300 or 900 pulses at low frequency (1 Hz) stimulation (LFS) were applied to the slices. LTP was induced by two sequential high-frequency stimulations (HFS: 100 Hz for 1 s, then repeated after 20 s). All LTD/LTP values represent fEPSP slopes measured 50 min after the conditioning stimulus unless stated otherwise. Two-tailed Student's *t* test and one-way analysis of variance (ANOVA) were used to determine statistical significance.

Whole-cell recordings were made from the soma of visually identified pyramidal neurons located in CA1 of the hippocampus. Patch pipettes (5–7 M Ω) were filled with an internal solution containing (in mM) 110 Cs-gluconate, 20 CsCl, 10 HEPES, 4 NaCl, 0.5 EGTA, 2 MgCl₂, 2 Na₂ATP, and 0.25 NaGTP, titrated with CsOH to pH 7.4. The excitatory postsynaptic currents (EPSCs) were recorded following stimulation of Schaffer collaterals ~150 μ m from the CA1 cell body with a bipolar electrode. AMPA-mediated EPSCs were recorded at a holding potential of –70 mV in the presence of 10 μ M bicuculline (BIC) and 50 μ M AP5. NMDA-mediated EPSCs were recorded at +45 mV in ACSF containing 20 μ M BIC and 10 μ M NBQX, or else at –70 mV in 0.1 mM Mg²⁺ ACSF containing BIC and NBQX. Series resistance was kept 15–30 M Ω and

monitored throughout each recording. Cells were excluded from data analysis if the series resistance changed by >20% during the course of the experiment. All patch-clamp experiments were performed at 24°C.

Glutamate Uptake Assay

Preparation of crude synaptosomal fractions and glutamate uptake assay were performed as described by Yang et al. (2005) with minor modifications. Briefly, hippocampal slices were treated for 2 hr with soluble A β , TBOA, or DMSO, then homogenized in 0.32 M sucrose, 1 mM EDTA, 4 mM Tris, and 10 mM glucose (pH 7.4) on ice. Homogenates were centrifuged at 1000 \times g for 10 min, and subsequent supernatants were spun at 9000 \times g for 10 min. Final pellets were resuspended in HEPES buffer solution to yield a protein concentration of 0.25 mg/ml. To avoid treatment washout, crude synaptosomes were treated with the same respective agents for 45 min at 37°C. Then, they were incubated with 10 nM [3 H]glutamate (20–60 Ci/mmol; Perkin Elmer, Boston, MA) and 30 μ M unlabeled glutamate for 5 min. The uptake was stopped by placing the samples on ice and spinning at 15,000 rpm for 10 min at 4°C. Pellets were washed and resuspended in ice-cold PBS. Glutamate uptake was counted on an LS6500 multipurpose scintillation counter. The absolute number of counts taken up varied from 500 to 40,000 counts per minute (cpm), with a background of 20 cpm.

Drug Treatments

Paired control and experimental hippocampal slices from a single mouse were maintained together in a single chamber, except during individual drug treatments. The following antagonists were purchased from Tocris (Ellisville, MO): DL-2-amino-5-phosphopentanoic acid (AP5), MK-801, MCPG, cyclothiazide, NBQX, (-)-bicuculline methiodide, SB203580, SB415286, Ryanodine, U73122, DL-threo- β -benzyloxyaspartic acid (TBOA) and dihydrokainic acid (DHK). Glutamic-pyruvic transaminase, pyruvic acid, Ifenprodil, and FK506 were purchased from Sigma (St Louis, MO).

SUPPLEMENTAL DATA

Supplemental Data include four figures and their legends and can be found with this article online at [http://www.cell.com/neuron/supplemental/S0896-6273\(09\)00387-0](http://www.cell.com/neuron/supplemental/S0896-6273(09)00387-0).

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